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Supplement to Safeguard: Calcium's Preventive Power in Preeclampsia Risk Pregnancy

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Abstract **Objectives:** Preeclampsia is a key cause of maternal morbidity and mortality worldwide, accounting for 34% of maternal deaths in Pakistan. Though the associated maternal-fetal complications can be reduced through preventive measures and strict monitoring. This study aimed to evaluate by comparing the effects of early calcium supplementation vs. delayed calcium supplementation on biomarkers (PlGF, sFlt-1, PP-13) in high-risk pregnant women for preeclampsia in Karachi, Pakistan. **Methods:** The study enrolled 150 pregnant women into a control and treatment group (75 each). The treatment group received calcium during the first trimester, while the control group began calcium supplementation at 12 weeks of gestation. Blood samples were collected at the outset and during follow-up for the measurements of serum biomarkers for preeclampsia [Human placenta growth factor (PlGF), Human soluble fms-like tyrosine kinase receptor 1 (sFlt-1), and Human Placental protein 13 (PP13)] by Enzyme-Linked Immune Sorbent Assay (ELISA) method. The collected data of serum biomarkers were tabulated and reported as Mean ± SD. Levels of serum biomarkers (PlGF, sFlt-1, and PP13) were analyzed by student's t-test, setting a significance level of ≤ 0.05 through SPSS 21. **Results:** The treatment group showed increased levels of PlGF and PP-13, while the control group had higher PlGF levels in the first trimester. In the second trimester, the calcium treatment group showed lower sFlt-1 levels and higher levels of PlGF and PP-13 than the control group. **Conclusion:** This study suggests early calcium supplementation could protect high-risk pregnant women, particularly in middle- and low-income countries.

Key Words Preeclampsia, Calcium, Biomarkers, Enzyme-linked Immunosorbent Assay

INTRODUCTION

Severe maternal and fetal morbidity and death can result from preeclampsia, a serious pregnancy condition marked by hypertension [1]. The complicated pathophysiology of preeclampsia includes many variables, such as maternal nutritional status, placental malfunction, and genetic susceptibility [2]. Calcium shortage is one of these causes that has become a serious problem, especially in communities with inadequate dietary calcium consumption. Early calcium supplementation may prevent preeclampsia, especially in high-risk women, according to research, which calls for a more thorough investigation of its possible advantages [3].

Blood pressure control and vascular health depend heavily on calcium. Increased blood pressure and vascular reactivity, which are critical in the development of hypertensive diseases during pregnancy, have been linked to low serum calcium levels [4]. According to studies, preeclamptic women frequently have much lower plasma calcium levels than pregnant women with normotension, which may indicate a connection between preeclampsia and calcium insufficiency [5,6]. The significance of having enough calcium during pregnancy was further supported by a comprehensive study that found that in populations with poor calcium consumption, calcium supplementation might lower the incidence of preeclampsia by about 55% [4,7].

A randomized controlled trial showed that women who received calcium and antioxidant supplementation during early pregnancy had a significantly lower prevalence of preeclampsia compared to those who received a placebo [3]. This finding highlights the potential of early intervention in high-risk populations, where timely calcium supplementation could mitigate the risk of developing hypertensive disorders. The timing and dosage of calcium supplementation are important factors influencing its

efficacy. Early calcium supplementation, particularly between weeks 8 and 12, may considerably reduce the risk of preeclampsia, according to the available data [3,8].

Additionally, the World Health Organization (WHO) and other medical associations have advocated for the use of calcium supplements in programs for prenatal care. The WHO recommends that pregnant women in regions with low dietary calcium intake take 1.5 to 2 grams of calcium daily to avoid preeclampsia [7,9]. The fact that different healthcare settings implement supplementing guidelines differently despite these recommendations highlights the need for effective ways to promote adherence to them [7,9].

The physiological mechanisms by which calcium inhibits preeclampsia include its modulation of vascular tone and endothelial function. Having enough calcium may help prevent the elevated vascular reactivity associated with preeclampsia because calcium is essential for the contraction and relaxation of smooth muscles [10,11]. The connection between calcium and other nutrients, such as vitamin D, has also been studied. According to some research, taking supplements of both vitamins may increase their ability to prevent hypertension [12].

An increasing amount of research supports the preventive potential of early calcium supplementation in high-risk females to avoid preeclampsia. The significance of managing calcium consumption in prenatal care is highlighted by the connection between blood pressure management and calcium levels, as well as the advantages of early supplementation. As our knowledge of the complex nature of preeclampsia grows, including calcium supplements in standard prenatal care may be a major step toward lowering the prevalence and severity of this dangerous illness.

Based on the above discussion, there is a need for a study evaluating the effects of early calcium supplementation versus delayed calcium supplementation on biomarkers (PlGF, sFlt-1, PP-13) in high-risk pregnant women at risk for preeclampsia in our region. It is hypothesized that initiation of calcium intake in the initial weeks of pregnancy will reduce preeclampsia and associated complications. So the present study aims to compare the outcomes of calcium supplementation started in early gestation and in the second trimester to observe the preeclampsia progression by measuring serum biomarkers in females with a high risk of developing high blood pressure during pregnancy.

METHODS

It was a prospective case-control study conducted from June 1, 2020, to May 31, 2024, in a tertiary care hospital located in Karachi, Pakistan. Initially, information from all the pregnant women visiting their first antenatal checkup consecutively in the obstetric OPD was collected. Finally, 150 pregnant women were included in the study following the inclusion criteria. The sample size was calculated by OpenEpi, with a confidence interval of 95% and an Odds ratio of 0.35 and 19% of prevalence in our country, followed by screening for risk factors for developing preeclampsia. We included singleton pregnant women who had risk factors analyzed by a questionnaire designed according to the National Institute for Health and Care Excellence (NICE) for developing preeclampsia. We excluded the woman presenting with chronic hypertension, cardiovascular and renal diseases, and poor compliance with taking calcium supplements. Written informed consent has been taken from all included participants.

We divided the 150 pregnant women into two groups. For 75 women labelled as treated cases with calcium. We started calcium supplementation containing 500mg of elemental calcium as calcium carbonate (two tablets with each of their morning and evening meals immediately from their first antenatal visit until delivery). They were also asked to drink at least six glasses of fluid per day. Other (75) women, labelled as control, started calcium at 12 weeks of pregnancy. We took a detailed history of calcium from all selected patients.

Measurements of serum biomarkers for preeclampsia (Human placenta growth factor (PlGF), Human soluble fms-like tyrosine kinase receptor 1 (sFlt-1), and Human Placental protein 13 (PP13)) were performed at the baseline (first trimester) and follow-up (in the second trimester). For that purpose, peripheral venous blood samples were collected from all included women in the study, and the serum fraction was separated by centrifugation and stored at -70°C. The sFlt-1, PlGF, and PP13 were analyzed by Enzyme-Linked Immune Sorbent Assay (ELISA) (Bioassay Technology Laboratory, Shanghai, China) as per the manufacturer's instructions.

The collected data of serum biomarkers were tabulated and reported as Mean ± SD. Levels of serum biomarkers (PlGF, sFlt-1, and PP13) between the control and treated groups, as well as in the control and treated groups in the first and second trimesters of pregnancy, were analyzed by Student's t-test, setting a significance level of ≤ 0.05 through SPSS 21.

This study has been approved by the Advanced Studies Research Board (ASRB) as a part of a postgraduate study (PhD). It is also approved by the Institutional Bioethics Committee. (IBC KU- 419/2024)

RESULTS

Baseline characteristics of both the control and early calcium-treated patient groups were measured and presented in Table 1.

Table 1: Demographic characteristics of control and early calcium-treated patients

Characteristics	Control (n=75)	Treated (n=75)
Age (Years)	25.96±4.24	28.57±5.14
Pre-Pregnancy Weight (Kg)	62.67±12.81	65.88±13.85
Weight at Enrolment (Kg)	65.37±11.64	67.55±13.26
Height (cm)	160.7±5.61	159.2±16.01
BMI	25.57±4.94	26.71±5.26
Waist/Hip ratio	0.827±0.05	0.778±0.03

The ± values are Mean±SD, Abbreviation: BMI: Body Mass Index

Table 2: Comparison of different biomarkers of preeclampsia between the control and Calcium-treated group during the first and second trimesters of pregnancy

Biomarkers	First Trimester		p-value	Second Trimester		p-value
	Control (C1)	Treated (T1)		Control (C2)	Treated (T2)	
PlGF (pg/ml)	203.92±19.3	195.08±23.6	0.009	163.72±16.2	461.23±13.8	0.005
sFlt-1 (pg/ml)	2.6±0.5	2.8±0.4	0.12	33.45±2.5	11.21±1.9	0.001
PP-13 (ng/ml)	201.2±24.6	199.5±32.4	0.65	634.6±29.4	1172.4±42.7	0.01

All values are Mean ± SD, p-value is significant at <0.05, Abbreviations: C1=Control in first trimester, T1: Treated in first trimester, C2=Control in second trimester, T2: Treated in second trimester, PlGF: Placental growth factor, PP-13: Placental Protein-13, sFlt-1: soluble fms-like tyrosine kinase-1

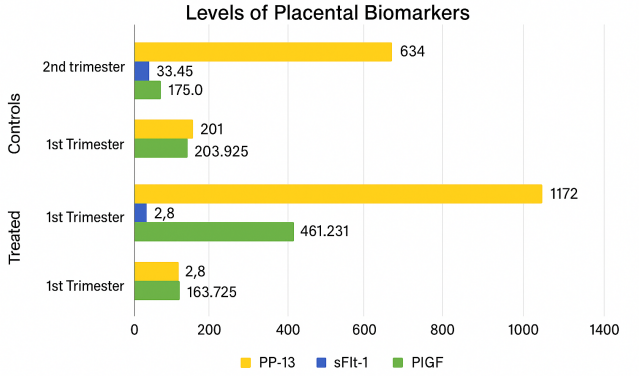

Figure 1: Comparison of PlGF levels (pg/ml), sFlt-1 levels (pg/ml) and PP-13 levels (ng/ml) during first and second trimesters in the control and calcium-treated group

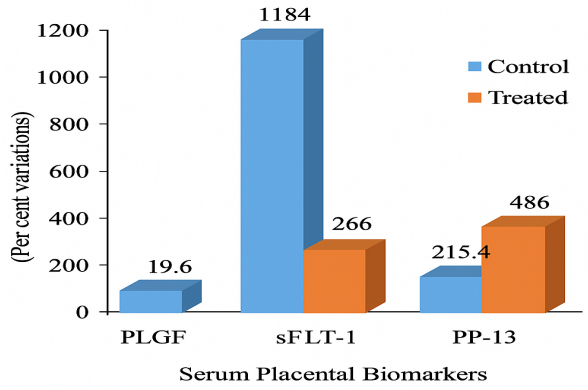

Figure 2: Per cent Variation of serum biomarkers of preeclampsia from Baseline to the second trimester between control and calcium treated group

In this study, during the first trimester, PlGF levels were found to be higher in the control group as compared to the treated group, with a p-value of <0.009. While other markers (sFlt-1 and PP-13) show no statistically significant differences between the groups. In the second trimester, both PlGF and PP-13 were found to be higher in the early calcium-treated group as compared to the control group, with significant p-values of 0.005 and 0.01, respectively. Whereas sFlt-1 levels were lower in the treated group as compared to the control, with a significant p-value of 0.001 (Table 2).

We also compared all three biomarkers in the control and early calcium-treated groups in the first and second trimesters separately. Figure 1 demonstrates the serum PlGF level found to be high in the second trimester as compared to the first trimester in the treated group, with a p-value of 0.002. Whereas, in the control group, its level was lower in the second trimester than in the first trimester, but this difference was not statistically significant (p=0.06). Serum sFlt-1 level was found to be very high in the second trimester as compared to the first trimester in the control group, with a p-value of 0.0001. Whereas, in the treated group, its level was also higher in the second trimester than in the first trimester, with a statistically significant level of p=0.01, but much lower than in the control group. The serum PP-13 level was found to be very high in the second trimester, as compared to the first trimester in the treated group, with a p-value of 0.001. Whereas, in the control group, its level was also higher in the second trimester than in the first trimester, with a statistically significant level of p=0.01, but not much higher than the treated group.

Figure 2 expresses the percent variation of different serum biomarkers between the control and early calcium-treated groups from baseline to the second trimester. From baseline to the second trimester, the PlGF level went down to -19.6 in controls, while it increased to 135 in the treated group. sFlt-1 level went up to 1184 in the control group as compared to the treated group, which is 266. The PP-13 level went up to 486 in the treated group, which was higher than the control group, that was 215.4.

DISCUSSION

Recent studies have focused a lot of emphasis on the preventive potential of early calcium supplementation in avoiding preeclampsia in high-risk females. The health of the mother and the fetus is seriously at risk from preeclampsia, a dangerous pregnancy condition marked by proteinuria and hypertension. Numerous studies have examined the connection between calcium consumption and the prevalence of preeclampsia; the results indicate that sufficient calcium supplementation may help reduce these risks, especially in populations with poor dietary calcium intake.

Poor calcium consumption, poor vitamin D intake, and preeclampsia are all linked, according to a comprehensive review by Bryce et al., which also noted that calcium supplementation by itself has been proven to significantly lower the risk of preeclampsia and preterm birth (PTB) [13]. This research emphasizes the value of calcium over vitamin D in preventing preeclampsia. The effectiveness of supplementation is dependent on baseline calcium intake levels, as a study pointed out that although calcium

supplementation is advantageous, it might not produce noticeable effects in people who already meet their calcium requirements [14]. This implies that groups with known inadequacies should be the focus of focused supplementing initiatives.

Researchers have further bolstered the effectiveness of calcium supplementation by showing that early calcium supplementation in high-risk women may reduce the incidence of preeclampsia [3]. The particular effects of calcium during the early stages of pregnancy, which are crucial for placentation and the eventual development of preeclampsia, were to be evaluated in this multicenter, double-blind, randomized controlled experiment. Although high-dose calcium supplementation starting at 20 weeks of gestation did not significantly lower preeclampsia rates, the data showed that the timing of supplementation is still an important factor for future research [3].

According to a systematic review and meta-analysis in a related study, taking calcium supplements along with low-dose aspirin successfully delays the onset of preeclampsia, which lowers the risk of preterm delivery and postpartum hemorrhage [11]. This combination medication demonstrates how treating several risk factors linked to preeclampsia can have synergistic benefits. Moreover, the results of another study supported the idea that a higher calcium consumption is negatively correlated with preeclampsia incidence, indicating that dietary practices have a big impact on results [15].

Supplementing with calcium has effects that go beyond the immediate results of pregnancy. Pregnant women in underdeveloped nations frequently have nutritional inadequacies, especially calcium, which can lead to negative consequences, including preeclampsia and intrauterine growth retardation [15]. This emphasizes the necessity of public health campaigns to increase pregnant women's dietary calcium consumption, especially in areas where shortages are prevalent.

Furthermore, research has indicated that the time and amount of calcium supplementation are important variables that affect how beneficial it is. A possible change in recommendations to account for different dietary practices and calcium intake levels is indicated: that lower calcium dosages may be equally helpful as larger doses in avoiding preeclampsia [16]. The WHO's recommendations for calcium supplementation, which prescribe 1.5 to 2 grams daily for women in areas with inadequate calcium consumption, make this more pertinent [17].

The link between calcium levels and blood pressure management is also interesting. A comprehensive study indicated a substantial reduction in preeclampsia risk related to calcium supplementation, particularly in women with poor dietary calcium consumption [18]. This is consistent with research, which highlighted the role of calcium in controlling blood pressure and avoiding pregnancy-related hypertension problems [19]. Notably, there is also a connection between blood pressure management and calcium levels. A comprehensive study showed that taking calcium supplements significantly decreased the incidence of preeclampsia, especially in women who had poor dietary calcium consumption [18]. This is consistent with other research, which highlighted its significance [19].

With the best of our research, this is the only study that was carried out in Pakistan. The study emphasized the role of early calcium supplementation for all pregnant women, especially those who are at risk of developing preeclampsia. The health policy of the Government of Pakistan, as well as poor socio-economic countries, should include early calcium supplementation for all high-risk pregnant women during their antenatal care. The limitations of the study were that since the majority of the study's participants received prenatal care, they may not be entirely representative of pregnant women who do not seek healthcare during pregnancy. There were no long-term neonatal outcomes noted. Further, it was a single centered study. There is a need for a multi-centered study to include both the government and private sector hospitals to cover the entire community.

CONCLUSIONS

Preeclampsia and its complications can be avoided by starting calcium therapy early in a woman's pregnancy, especially in those who are malnourished or have inadequate nutritional intake, but further multicenter studies are required" to maintain balance.

Institutional Review Board statement

This study has been approved by the Advanced Studies Research Board (ASRB) of the University of Karachi, Pakistan, as a part of a postgraduate study (PhD). It is also approved by the Institutional Bioethics Committee, University of Karachi. (IBC KU-419/2024)

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Conflicts of Interest

There were no funding sources and no conflicts of interest.

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